ANALYTICAL BIOCHEMISTRY **247,** 272–278 (1997)
ARTICLE NO. AB972069

Method for the Collection and Assay of Volatile Organic Compounds in Breath

Michael Phillips

Menssana Research, Inc., 1 Horizon Road, Suite 1415, Fort Lee, New Jersey 07024; Department of Medicine, St. Vincent's Medical Center of Richmond, Staten Island, New York 10310-1699; and New York Medical College, Valhalla, New York

Received October 23, 1996

Breath testing for volatile organic compounds (VOCs) provides an intrinsically safe method for investigating human metabolism. An improved breath-collecting apparatus (BCA) is described which was acceptable to patients, simple to use, highly sensitive, and free from chemical contamination. VOCs in 10.0 L alveolar breath and 10.0 L room air were collected onto adsorptive traps. Using automated instrumentation, VOCs were thermally desorbed and assayed by gas chromatography/mass spectroscopy. Twenty normal volunteers were studied, and the alveolar gradient (concentration in breath minus concentration in air) was determined for the most abundant VOCs. A total of 1259 VOCs were observed and tentatively identified in the breath of normal subjects. The mean alveolar gradients were positive in 461 VOCs and negative in 798 VOCs. The method provided a sensitive and convenient assay for breath VOCs and permitted tentative determination of their origin from either inside or outside the body.

Breath tests open a unique window onto the composition of the blood. Normal human alveolar breath contains a large number of volatile organic compounds (VOCs)[1] derived from the blood by passive diffusion across the pulmonary alveolar membrane (1–5). Breath testing for VOCs is intrinsically safe and noninvasive, and might offer a new approach to the early diagnosis or evaluation of several common disorders including lung cancer (6, 7), heart disease (8, 9), exposure to environmental toxins (4, 10), schizophrenia (11, 12), malnutrition (13), rheumatoid arthritis (14), *Pneumocystis carinii* pneumonia (15), and inflammatory bowel disease (16, 17).

[1] Abbreviations used: volatile organic compounds, VOCs; breath collecting apparatus, BCA.

However, breath testing is technically difficult because most breath VOCs are excreted in nanomolar (10^{-9} M) or picomolar (10^{-12} M) concentrations. Since these levels are too low for detection by most instrumentation, breath VOCs must be concentrated prior to assay. This, in turn, requires special apparatus for the collection and concentration of breath. Several ad hoc methods have been described, utilizing cold trapping (18), adsorptive binding (19), and chemical trapping (19) to capture the VOCs in the breath while allowing the free passage of the nitrogen and oxygen (1).

Progress in breath testing has been limited by the lack of generally accepted and standardized methodology for the collection and analysis of breath VOCs. This report describes a new breath-collecting apparatus (BCA) which was developed from an earlier prototype (2). The BCA is portable and "user-friendly," and provides samples which can be analyzed by standard assay techniques.

MATERIALS AND METHODS

Structure of the BCA. The BCA (Fig. 1) was developed by the author (at Menssana Research, Inc.) and constructed by De Vilbiss Health Care, Inc. (Somerset, PA). It comprises a portable microprocessor-controlled device which collects alveolar breath onto an adsorbent tube; the duration and flow rate of breath collection are controlled by settings on the front panel. In practice, the subject wears a nose clip and breathes in and out through a disposable mouthpiece containing inlet and outlet flap valves. The BCA was designed to fulfill the following requirements:

(1) Subject comfort: the subject breathes into a wide-bore tube (approx 1 in. diameter) which presents very little resistance to expiration. The person providing the breath sample experiences no discomfort and encounters virtually no resistance while breathing into the device. Hospitalized patients, including some who were

0003-2697/97 $25.00

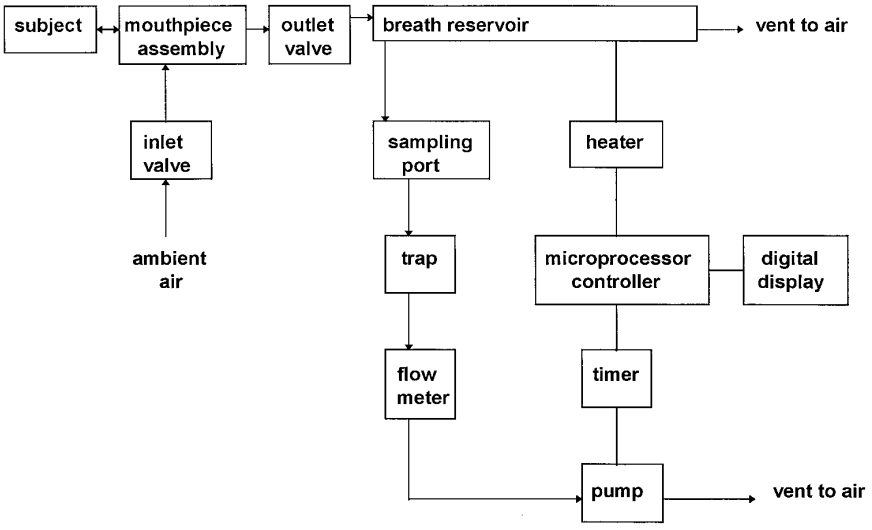

FIG. 1. The breath collecting apparatus (BCA). Arrows indicate the movement of air and breath. The mouthpiece assembly, outlet valve, and inlet valve comprise a single disposable unit. The subject wears a nose clip and respires in and out through the mouthpiece assembly. The breath reservoir, a heated tube, contains a column of alveolar breath proximal to the outlet valve; dead-space air is downstream and not sampled. VOCs are captured in the trap, a stainless-steel tube containing adsorbent resins. The volume of alveolar breath sampled in the trap is controlled by varying the flow rate through the flow meter and the duration of sampling.

severely ill, were able to provide breath samples without discomfort.

(2) Subject safety: Using a sterile disposable mouthpiece with inlet and outlet valves, the subject inspires room air and expires into the instrument, so that there is no potential hazard of exposure to infection.

(3) Alveolar sampling: The breath sample was drawn principally from alveolar breath, not from dead-space breath in which no VOC interchange had occurred. The subject expires into a long tubular reservoir; with each expiration a column of breath enters into this reservoir with the dead space breath farthest downstream. Breath is removed from the reservoir at a sampling port close to the subject's mouth, i.e., from the upstream component of the column of breath which contains alveolar breath from deep in the lungs. The sample is withdrawn at a rate which ensures that the alveolar breath in the reservoir is not depleted before the next expiration is delivered. Dead space breath passes the sampling port for only a fraction of a second during each expiration, so that the withdrawn sample is composed almost entirely of alveolar breath.

(4) Freedom from chemical contamination: The device incorporated no structural components such as volatile adhesives which might outgas VOCs and contaminate the sample. The breath sample is ducted through stainless-steel tubing; the only plastic component is the disposable mouthpiece which is manufactured with a low content of plasticizer.

(5) Freedom from condensation: Breath is saturated with water; none should condense in the collecting apparatus since VOCs could be lost by partitioning into the aqueous phase. The reservoir tubing is heated to 40°C, in order to prevent any condensation within the system.

(6) Concentration of sample: The apparatus trapped and concentrated the VOCs contained in the alveolar breath, while allowing the nitrogen, oxygen, and carbon dioxide in the breath to escape. The sample is drawn from the sampling port through a trap containing adsorbent agents which trap VOCs while allowing most other components of breath to pass through unhindered. The trap may incorporate adsorbents such as resins or activated carbon.

(7) Control for VOCs in inspired air: Since a VOC in the breath may have originated in either the body or the inspired air, the collection method should permit determination of its source. In the earlier prototype, an attempt was made to supply the subject with a source of purified air (2). However, subsequent studies have demonstrated that it is technically difficult as well as unnecessary to provide a portable source of purified air which is devoid of VOCs in picomolar concentrations. Determination of alveolar gradient of a VOC (concentration in alveolar breath minus concentration in room air) was found to provide a simpler alternative approach. The alveolar gradient was generally positive for compounds manufactured in the body and negative for environmental pollutants (10, 21).

(8) User convenience: The breath-collecting appara-

tus was portable, simple to use in clinical and field settings, and did not require a high degree of training for the operator.

Use of the apparatus. The BCA operates from a standard AC electrical power source. The flow rate and duration of breath sampling are set on the control panel. A temperature control circuit operates a heater band surrounding the reservoir, maintaining it at 40°C. Sample collection time is displayed digitally in minutes and a flow meter controls the rate at which alveolar breath is sampled from the reservoir (in liters/minute).

Sorbent traps. Standard traps were employed for this research (Carbotrap, Supelco, Bellefonte, PA, containing 4.4 g Carbotrap C, 3.2 g Carbotrap, and 2.0 g Carbosieve SIII). These traps were found to provide high reproducibility and sensitivity. The traps were reusable. Prior to use, each trap was cleaned by heating to 340°C for 3 h in a flow of helium (35 ml/min) in an automated apparatus (5100 thermal trap conditioner, Tekmar Co., Cincinnati, OH). Two of every batch of 12 traps were assayed by GC/MS as described below for quality assurance that they were chemically clean.

Breath collection method. Subjects sat in front of the BCA wearing a nose clip, breathing in and out through the mouthpiece which was adjusted to a comfortable position. Breath samples were collected for 5.0 min at a rate of 2.0 L/min (although smaller or larger samples could be collected if required). A sample of ambient room air was collected in a similar fashion immediately before or after the breath collection. During pilot studies, collections were also made onto a second trap connected in series, but no breakthrough of VOCs to the second trap was detected when these collection parameters were employed.

Human study. We studied 10 males (mean age, 30.9 years; SD = 3.2) and 10 females (mean age, 35.3 years; SD = 3.9). These normal volunteers were recruited from the medical and nursing staff of St. Vincent's Medical Center of Richmond. Samples were collected between 0700 and 1100 following an overnight fast. The research was approved by the Institutional Review Board of St. Vincent's Medical Center of Richmond.

Breath assay method. In summary, the VOC sample was thermally desorbed from the adsorptive trap, concentrated by two-stage cryofocusing, then analyzed by GC/MS. Breath VOCs were thermally desorbed using an Aerostar 6000 desorber attached to a 6016 Aerotrap autosampler which allowed the sequential desorption of up to 16 traps (Tekmar Co.). Samples were purged for 10.0 min with helium flowing at 10.0 ml/min and then heated to 250°C for 8.0 min. The desorbed VOCs were captured in two sequential cryotraps cooled to −150°C; the sample in the second cryotrap was heated to 250°C and injected into the GC/MS over 1.5 min. An HP 5890 Series II Plus GC (HP5MS column: 30 m, 0.25 mm diameter, 0.25-μm thickness, No. 19091S-433) with 5972 mass selective detector (Hewlett–Packard, North Hollywood, CA) was used with temperature programming: −5°C for 10.0 min, rising at 6.0 C°/min to 108°C and then at 30.0 C°/min to 258°C. VOCs were quantified by their area under curve and tentatively identified from a computer-based library (Wiley 120, using 70% criterion for quality of match).

Identification, quantitation, accuracy, and precision. Styrene was selected for study as a typical VOC observed in human breath. One peak in the breath chromatogram was tentatively identified as styrene by automated reference to a computer-based library of mass spectra. The elution time and mass spectrum of this peak was then compared to the elution time and mass spectrum obtained with pure styrene (S6450, Sigma Chemical Co., St. Louis, MO). A standard curve (relating area under peak to quantity of styrene) was obtained by loading adsorptive traps with vapor standards prepared by the method of Morris *et al.* (22). Accuracy and precision were determined by analysis of sets of five replicate samples loaded with a known amount of styrene vapor standard.

RESULTS

Detection of Breath VOCs

A typical chromatogram yielded 150–200 different VOCs in the breath (Fig. 2). The cumulative number of different VOCs increased with the number of subjects studied (Fig. 3). A total of 1259 VOCs were observed; the alveolar gradients were positive in 461 VOCs and negative in 798 VOCs. The most abundant VOCs with positive and negative alveolar gradients are shown in Table 1, with their relative abundance.

Identification, Quantitation, Accuracy, and Precision

Chromatograms of styrene vapor standards exhibited a retention time (20.67 min) and mass spectrum similar to those observed in peaks tentatively identified as styrene in chromatograms of breath. Breath styrene was quantified from the linear standard curve (r^2 = 0.91); replicate samples exhibited some variation eventually traced to instrumental malfunction; accuracy of 95% and precision (coefficient of variation) of 11.7% were the highest values observed.

DISCUSSION

Interpretation of breath VOC data is a rapidly evolving field. Measurement of exposure to environmental toxins is the best documented and least controversial application, while monitoring of breath pentane and ethane has been advocated as a marker of intracellular free radical activity causing the peroxidation of polyunsaturated fatty acids (23). With the advent of instru-

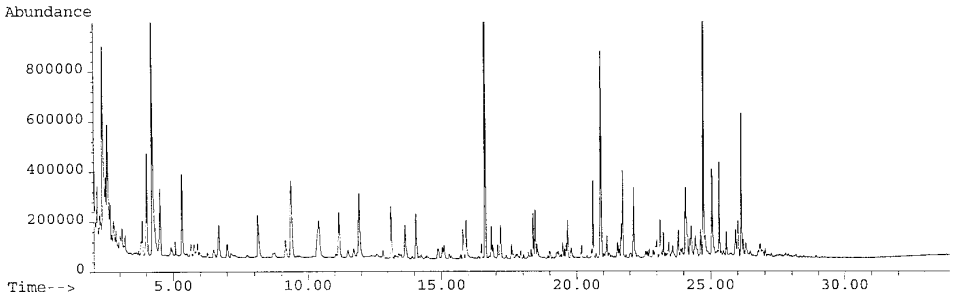

FIG. 2. Chromatogram of breath VOCs in a normal subject.

mentation capable of detecting nanomolar and picomolar concentrations of VOCs in the breath, it has become apparent that many of these compounds can also be detected in normal room air. Pentane, for example, has been observed in breath and air in concentrations sufficiently similar to provoke speculation as to whether or not the compound was manufactured in the body (24, 25). Therefore, a highly sensitive breath assay needs to distinguish the signal (VOC manufactured in the body) from noise (VOC pollution in the ambient air). Two different experimental approaches to this problem have been advocated: either reduce the background noise to zero, or else subtract the background noise from the breath signal.

In systems designed to reduce background noise to zero, human subjects are supplied with ultrapure breathing air derived either from a cylinder or from room air filtered through a bed of activated carbon. No such system has yet proved completely satisfactory because of the technical difficulties of supplying breathable air which is completely free of detectable VOCs in picomolar concentrations.

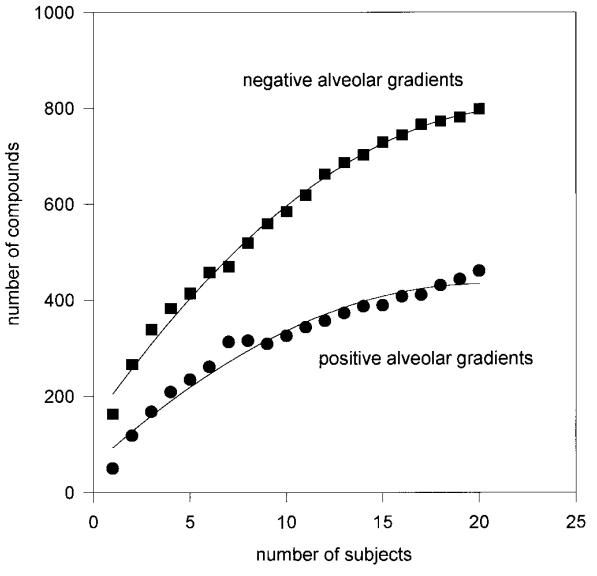

FIG. 3. Cumulative number of different VOCs observed. The number of VOCs increased with the number of subjects studied. Lines of best fit: $y = -0.97x^2 + 37.2x + 56.2$ (VOCs with positive alveolar gradients); $y = -1.26x^2 + 57.4x + 148.9$ (VOCs with negative alveolar gradients).

An alternative experimental approach has been proposed by the author in which VOCs are assayed in both alveolar breath and in room air (10, 12, 21, 25, 26). The difference between the two concentrations, termed the alveolar gradient, provides an indication of whether a particular VOC is endogenous or exogenous in origin. Generally, the alveolar gradient is positive for VOCs manufactured in the body since more is excreted in the breath than is inspired from the air. Conversely, the alveolar gradient is negative for air pollutants which are excreted or metabolized by extrapulmonary pathways. There are a number of advantages to this approach: First, it frees investigators from laborious (and generally fruitless) attempts to provide subjects with VOC-free air. Second, it indicates whether a breath VOC was endogenous or exogenous in origin. Third, it provides a new indicator of individual differences in disposing of air pollutants from the body. Fourth, it permits the design and construction of a BCA which is portable and suitable for field use.

A potential disadvantage of this approach is that determination of alveolar gradient requires the subject to be in equilibrium with room air before the sample is collected. The calculated alveolar gradient of a particular VOC will be erroneous if the subject has not equilibrated with room air, which might occur if there has been exposure to a VOC with a long residence half-life in the body. In this study, all subjects had been breathing room air for at least 1.0 h in the same environment where the breath collection was performed.

The alveolar gradients of breath pentane (25), carbon disulfide (10), and isoprene (26) have been previously studied in normal subjects. The alveolar gradients of pentane and carbon disulfide were distributed about approximately bell-shaped curves with a mean of slightly less than zero, suggesting that these VOCs are pollutants of room air which are not normally manufac-

TABLE 1

Breath VOCs in Normal Subjects

	Relative abundance (%)	Retention time (min)
VOCs with positive alveolar gradients		
Isoprene	48.60	2.42
1,2-Pentadiene	15.00	2.55
Acetone	14.59	2.35
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (*R*)-	8.43	22.77
dl-Limonene	2.31	26.06
1-Propene, 3-(methylthio)-	2.07	12.51
1-Propene, 1-(methylthio)-, (*cis*)-	1.58	14.61
Propane, 1-(methylthio)-	0.69	13.85
Hexane, 2,2,4-trimethyl-	0.61	17.17
2-Deutero-2-methylpropane	0.54	3.56
1-Propanol	0.51	4.27
1-Propene, 1-(methylthio)-, (*trans*)-	0.45	14.82
Xylene	0.42	21.54
2-β Pinene	0.35	24.33
Heptane, 3,5-dimethyl-	0.30	19.81
Nonanal	0.26	14.92
Silanol, trimethyl-	0.26	4.79
Benzene, 1,3,5-trimethyl-	0.23	24.98
α-Terpinolene	0.21	27.88
Cyclohexane,1,2-dimethyl-	0.21	17.93
Benzene, chloro-	0.20	19.99
Benzene, (1-methylethenyl)-	0.18	24.20
Propanamide	0.18	8.14
Ethene, tetrachloro-	0.15	17.99
Cyclobutanol	0.14	5.92
Isooctane	0.13	11.91
2,4-Dimethyl-1-heptene	0.12	19.66
Disulfide, dimethyl	0.11	15.45
Piperazine	0.11	9.43
Methanamine, *N*-methyl-	0.10	2.64
Pyrrolidine	0.09	16.65
α-Thujene	0.08	25.36
Furan, 2-methyl-	0.08	5.87
1-Pentene, 4-methyl-	0.08	7.72
Pentane, 3-ethyl-2,2-dimethyl-	0.08	4.43
Cyclohexane, 1,1,2-trimethyl-	0.07	19.88
Acetamide, 2-cyano-	0.07	11.96
Octane, 2,6-dimethyl-	0.06	23.89
[1′-^{13}C]Octyne	0.06	19.23
2-Heptenal, (*trans*)-	0.06	20.57
Heptane, 2,4-dimethyl-	0.05	16.58
[^{1}H]Pyrrole, 2,5-dihydro-1 nitroso	0.05	18.15
1-Butanol, 2-ethyl-	0.03	16.61
Cyclopentane, 1,2,3-trimethyl-, (1, α; 2, α; 3, β)-	0.02	15.74
2-Pentanone	0.02	12.91
Heptane, 2,2,4-trimethyl-	0.02	21.41
Dodecane	0.02	25.31
1-Pentene, 3,4-dimethyl-	0.01	14.35
N-Octan-3-ene	0.01	18.49
Cyclopentene, 1,5-dimethyl-	0.01	16.20
VOCs with negative alveolar gradients		
Butane, 2-methyl-	8.22	3.14
Benzene, methyl-	6.30	16.30
Propane, 2-methoxy-2-methyl-	6.08	4.22
Pentane	5.34	2.55
Benzene, 1,2-dimethyl-	4.62	20.91
Benzene, 1,2,3-trimethyl-	3.74	24.10
Benzene, 1,3-dimethyl	3.74	20.23
2-Propanol	2.84	2.58
Decane	2.75	24.79
Ethanol	2.55	2.32

TABLE 1—*Continued*

	Relative abundance (%)	Retention time (min)
Benzene, 1,4-dimethyl-	2.54	21.54
Cyclohexane, methyl-	2.43	13.73
Hexanal	2.36	17.80
Benzene, 1,2,4-trimethyl-	2.34	24.90
Nonane	2.25	22.12
Hexane, 2-methyl-	2.15	10.87
Nonane, 3-methyl-	2.04	23.90
Octane	1.97	18.52
Pentane, 2-methyl-	1.91	3.99
Benzene, 1-ethyl-2-methyl-	1.71	24.28
Undecane	1.68	28.23
Heptane, 3-methyl-	1.54	17.81
Benzene, ethyl-	1.53	20.22
Benzene	1.42	9.21
Hexane, 3-methyl-	1.41	10.79
Methane, dichloro-	1.39	3.28
Benzene, (1-methylethyl)-	1.31	22.91
Hexane	1.26	5.85
Pentane, 3-methyl-	1.22	4.46
Benzene, 2-ethyl-1,4-dimethyl-	1.19	27.68
1-Butene, 2-methyl-	1.17	4.86
1,3-Pentadiene, (*cis*)-	1.15	2.82
Ethene, trichloro-	1.14	12.88
Heptane	1.12	12.85
Cyclopentane, 1,2-dimethyl-3-(1-methylethyl)-	1.04	24.94
[^{1}H]Indene, 2,3-dihydro-	1.03	25.27
Butane, 2,3-dimethyl-	0.98	3.91
Cyclohexane, 1-ethyl-4-methyl-, (*cis*)-	0.94	21.87
2-Pentanone, 4-methyl-	0.91	15.91
Cyclopentane, methyl-	0.91	6.57
Benzene, propyl-	0.88	23.02
Decane, 4-methyl-	0.85	24.53
α-Pinene, (−)-	0.80	22.23
Octane, 3-methyl-	0.79	21.17
Cyclohexane, ethyl-	0.77	19.24
Heptane, 2-methyl-	0.76	16.47
Ethane, 1,1,1-trichloro-	0.76	7.98
Pentanal	0.75	12.87
Benzene, 1-methyl-2-(1-methylethyl)-	0.72	27.47
Acetic acid, butyl ester	0.71	19.30

Note. Ranking of the breath VOCs with the greatest positive or negative alveolar gradients. Chemical structures were tentatively identified from a computer-based library of mass spectra. The relative abundance was determined as the mean alveolar gradient of a particular VOC divided by the total mean alveolar gradient of the 50 most abundant VOCs. The VOCs with negative alveolar gradients were present in at least 50% of subjects, while the VOCs with positive alveolar gradients were present in at least 16.7% of subjects.

tured in the bodies of most individuals. However, these studies also revealed subgroups of apparently normal individuals in whom the alveolar gradients were positive; i.e., a minority appeared to be manufacturing pentane, carbon disulfide, and/or isoprene endogenously. The significance of this observation is still unknown, and it is possible that a positive alveolar gradient may indicate a metabolic abnormality. There are reports of increased breath concentrations of pentane in patients with inflammatory bowel disease (16), rheumatoid arthritis (14), and schizophrenia (11).

The modern era of breath analysis commenced with the pioneer work of Pauling *et al.* in 1971 (27). They collected human breath volatiles by blowing through a stainless-steel tube chilled in isopropyl alcohol–dry-ice bath. The sample was heated then analyzed by gas chromatography. They observed around 250 different VOCs in the sample, but did not report any chemical identification of individual VOCs. In their apparatus, the breath donor expired into a narrow-gauge tube, which would probably have required a considerable respiratory effort. Since then, advances in technology have made the collection and analysis of breath VOCs considerably more convenient. The BCA described here is

a low-resistance system because the breath reservoir is a wide-bore tube open to the air, so that even debilitated patients and those with severe respiratory disease may donate breath samples without discomfort. In addition, the capture of VOCs onto sorbent traps rather than by cold-trapping has facilitated the collection of breath samples in the field. Laboratory analysis has been facilitated by the availability of automated instrumentation for thermal desorption, separation, and identification of VOCs in the sample, thereby permitting a much greater throughput of samples.

The BCA employed in this study was found to be convenient for both the breath donor and the operator of the instrument. In ongoing clinical studies, samples have been collected from more than 200 volunteers, none of whom has complained of discomfort while donating a breath sample. Operators were able to collect breath samples which were technically satisfactory after only a brief period of training.

This study has generated a larger data base of breath VOCs than has been previously reported. It is not yet known if any VOCs which are present in the breath are not observed with this method. The manufacturers of the adsorptive traps claim that they should capture virtually all VOCs, polar and nonpolar, from C1 to C12. However, it is possible that some polar VOCs may be lost in the automated thermal desorption apparatus during the process of water extraction from the sample. Previous studies have shown that the number of different VOCs in human breath grows larger as more subjects are studied (3, 6). While the total number of different VOCs in human breath is still unknown, the data in Fig. 3 suggest that the total number of endogenous VOCs may not be much greater than 400 even when larger numbers of subjects are studied. However, the source and physiological significance of most of these VOCs is still unknown. This presents both a problem and an opportunity to researchers. On the one hand, it presents a problem because a chromatogram of human breath generates a large amount of data about VOCs whose origin, significance, and normal concentration range are still unknown. Initial attempts to correlate a disease with a candidate VOC marker or combination of markers in the breath must often be made empirically. On the other hand, it also presents an opportunity to investigate human metabolic pathways in greater detail. Should a correlation between a breath VOC and a particular disease be consistently observed, this may present a unique opportunity for better understanding of the biochemical basis of that disease. In addition, breath testing might offer a new technology for diagnosis of diseases in their earlier and more treatable stages. The method of breath collection and analysis described here may provide a useful new tool to advance this research.

REFERENCES

1. Phillips, M. (1992) *Sci. Am.* **267,** 74–79.
2. Phillips, M., and Greenberg, J. (1992) *Clin. Chem.* **38,** 60–66.
3. O'Neill, H. J., Gordon, S. M., O'Neill, M. H., Gibbons, R. D., and Szidon, J. P. (1988) *Clin. Chem.* **34,** 1613–1618.
4. Raymer, J. H., Thomas, K. W., Pellizzari, A. B., Whitaker, D. A., Cooper, S. D., Limero, T., and James, J. T. (1994) *Aviat. Space. Environ. Med.* **65,** 353–360.
5. Raymer, J. H., Thomas, K. W., Cooper, S. D., Whitaker, D. A., and Pellizzari, E. D. (1990) *J. Anal. Toxicol.* **14,** 337–344.
6. Gordon, S. M., Szidon, J. P., Krotoszynski, B. K., Gibbons, R. D., and O'Neill, H. J. (1985) *Clin. Chem.* **31,** 1278–1282.
7. Preti, G., Labows, J. N., Kostelc, J. G., Aldinger, S., and Daniele, R. (1988) *J. Chromatogr. B Biomed. Appl.* **432,** 1–11.
8. Weitz, Z. W., Birnbaum, A. J., Sobotka, P. A., Zarling, E. J., and Skosey, J. L. (1991) *Lancet* **337,** 933–935.
9. Mendis, S., Sobotka, P. A., and Euler, D. E. (1995) *Free Radicals Res.* **23,** 117–122.
10. Phillips, M. (1992) *Int. Arch. Occup. Environ. Health* **64,** 119–123.
11. Kovaleva, E. S., Orlov, O. N., Tsutsul'kovskaia, Mia, Vladimirova, T. V., and Beliaev, B. S. (1989) *Zh. Nevropatol. Psikiatr. Im. SS Korsakova* **89,** 108–110.
12. Phillips, M., and Greenberg, J. (1993) *J. Clin. Pathol.* **46,** 861–864.
13. Lemoyne, M., Van Gossum, A., Kurian, R., and Jeejeebhoy, K. N. (1988) *Am. J. Clin. Nutr.* **48,** 1310–1315.
14. Humad, S., Zarling, E., Clapper, M., and Skosey, J. L. (1988) *Free Radicals Res. Commun.* **5,** 101–106.
15. Phillips, M., Clarkson, A. B., Merali, S., Phillips, A., and Greenberg, J. (1994) *Clin. Res.* **42,** 459A.
16. Kokoszka, J., Nelson, R. L., Swedler, W. I., Skosey, J., and Abcarian, H. (1993) *Dis. Colon Rectum* **36,** 597–601.
17. Sedghi, S., Keshavarzian, A., Klamut, M., Einzhamer, D., and Zarling, E. J. (1994) *Am. J. Gastroenterol.* **89,** 2217–2221.
18. Dannecker, J. R., Jr., Shaskan, E. G., and Phillips, M. (1981) *Anal. Biochem.* **114,** 1–7.
19. Phillips, M., and Greenberg, J. (1987) *Anal. Biochem.* **163,** 165–169.
20. Rogers, W. K., Wilson, K. M., and Becker, C. E. (1978) *Alcohol Clin. Exp. Res.* **2**(4), 375–380.
21. Phillips, M., Greenberg, J., and Awad, J. (1994) *J. Clin. Pathol.* **47,** 1052–1053.
22. Morris, C., Berkley, R., and Bumgarner, J. (1983) *Anal. Lett.* **16,** 1585–1593.
23. Kneepkens, C. M. F., Lepage, G., and Roy, C. C. (1994) *Free Radicals Biol. Med.* **17,** 127–160.
24. Cailleux, A., and Allain, P. (1993) *Free Radicals Res. Commun.* **18,** 323–327.
25. Phillips, M., Sabas, M., and Greenberg, J. (1994) *Free Radicals Res. Commun.* **20,** 333–337.
26. Phillips, M., and Greenberg, J. (1995) *J. Clin. Pathol.* **48,** 979–980.
27. Pauling, L., Robinson, A. B., Teranishi, R., and Cary, P. (1971) *Proc. Natl. Acad. Sci. USA* **68**(10), 2374–2376.